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Original Article

Morphometric, meristic and genetic characteristics of Atlantic bonito (*Sarda sarda*) in the Black Sea

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Abstract
The Atlantic bonito (*Sarda sarda*) is a commercially important and popular species in the world as well as in Turkish fisheries sector. In this study, the morphologic and meristic features and genetic characteristics of Atlantic bonito in the Black Sea were examined. We found that most of the morphometric measurements in females were greater than males. Besides, 10 haplotypes were found for COI gene region, 3 haplotypes for 16s gene region and 4 haplotypes for the rhodopsin gene region. Genetic closure was determined in Tirebolu population for the first time.

Keywords: Atlantic bonito; Black Sea; genetic characters; *Sarda sarda*; morphometric and meristic

1 | INTRODUCTION

The Atlantic bonito *Sarda sarda* (Bloch, 1793) is the most known representative of the small tunas in the world and it is highly commercial for the industrial, artisanal and small-scale fishers (Bulatov *et al.* 2019). The Atlantic bonito is oceanodromous and pelagic-neritic species and widely distributed in five geographic areas; North and South America, northeast Atlantic (Scandinavia, Atlantic Europe, the Azores), Mediterranean Sea (including the Black and Adriatic Seas) and the Gulf of Guinea (Yoshida 1980; Riede 2004; Froese and Pauly 2021). This species lives in subtropical waters (12 – 27 °C) (Collette and Nauen 1983) at 80 – 200 m depth ranges (Maigret and Ly 1986). Atlantic bonito known as cannibalistic and generally feeds on schooling fish, squid, shrimp, small fish species (Collette 1986), small / big pelagic and demersal species (*Engraulis encrasicolus*, *Trachurus mediterraneus*, *Sprattus sprattus*, *Mullus barbatus*, *Gobius niger*, *Merlangius merlangus*, *Pomatomus saltatrix*, *Alosa immaculata*, *Parablennius tentacularis*) in the Black Sea (Genç *et al.* 2019). The reproduction for this species occurs during the spring and summer months, usually from May to July (Artüz 1957; Valeiras *et al.* 2008; Kahraman *et al.* 2014). The Atlantic bonito is a multiple spawner with asynchronous oocyte development and it releases eggs multiple times during the spawning season (Majorova and Tkacheva 1959; Rey *et al.* 1984). In the Mediterranean, spawning occurs in the Black and Marmara seas as main spawning areas (Yoshida 1980; Rey *et al.* 1984). The species already received attentions of the researchers and different aspects including its biometry, age, growth, mortality, migration and genetic differentiations have been studied (Demir 1964; Franicevic 2005; Ateş *et al.* 2008; Zorica and Sinovcic 2008; Cengiz 2013; Kahraman *et al.* 2014; Turan 2015; Turan *et al.* 2015, 2016; Petukhova 2020) but no one has fully defined its morphometric, meristic and genetic characteristics.

The biometric measurements are essential for a definition of species originating from different areas. Therefore, we aimed to determine the variation and definition

of morphometric characters by sexes of Atlantic bonito in the Black Sea. In addition to this, these results were supported by genetic characteristics for the compare all results.

2 | METHODOLOGY

One hundred and twenty Atlantic bonito (60 female and 60 male) individuals were sampled from the Black Sea between August and September 2020. The individuals were sampled by using trammel net (mesh size: 18 mm) and purse seine net (mesh size: 24 mm). The specimens were weighed and measured following the measuring scheme shown in Figure 1. All length measurements were made with a digital calliper, which is the nearest 1 mm precision. The morphometric results were considered statistically significant at $p < 0.05$. The data were analysed by Microsoft Excel 2019.

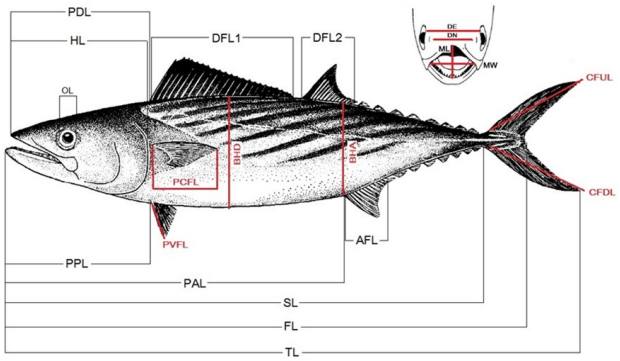

FIGURE 1 Morphometric measurements of *Sarda sarda* (modified from Fischer *et al.* 1987 by YY Kasapoglu).

For genetic studies, the caudal fin samples of species were collected from 3 different sampling stations (Sinop, n = 11; Tirebolu, n = 25; Trabzon, n = 34) and they were preserved in 98% ethanol. Total DNA isolation was performed using the QIAamp 96 DNA QIAcube HT commercial kit. The estimation of concentration and purity of DNA samples was made using Nanodrop (NanoDrop 8000, USA) by reading its optical density at 260 and 280 nm wavelengths. The mitochondrial DNA markers which are cytochrome c oxidase subunit I (COI), 16s rRNA and nuclear DNA marker Rhodopsin (rod) gene regions were used as potential markers for species identification and genetic diversity assessment. The mitochondrial DNA gene regions were amplified using primer pairs U.COI (5′ - TTCTCAACTAACCAYAAAGAYATYGG - 3′ and 5′ - TAGACTTCTGGGTGGCCRAARAAYCA - 3′) and barcoding 16Sar (5′ - CGCCTGTTTATCAAAAAACAT - 3′ and 5′ - CCGGTCTGAACTCAGATCACGT - 3′). PCR was prepared as a total volume of 20 µl of reaction solution that contained 10 µl of 2X Master mix (Qiagen), 1 µM of each primer (F and R), 90 – 150 ng of DNA and ddH_2O. The cycling conditions were as follows: 94°C for 5 minutes; then 35 cycles at 95°C for 1 minute, at 58°C for 90 seconds and 72°C for 1 minute; the final step 10 minutes at 72°C. The rhodopsin gene was amplified with primer pair Rod-F2 (5′ - AGCAACTTCCGCTTCGGAGAGAA - 3′) and Rod-R4n (5′ - GGAACTGCTTGTTCATGCAGATGTAGAT - 3′). PCR was performed at the same conditions for the COI gene and 16s rRNA gene reaction solution. The cycling conditions were as follows: 94°C for 5 minutes, then 35 cycles at 95°C for 1 minute, at 62°C for 90 seconds and 72°C for 1 minute, final step 10 minutes at 72°C. For the sequencing of all gene regions, the samples were purified with the BigDye v.3.1 Terminator Cycle Sequencing Kit on the ABI 3500 Genetic Analyser (Thermo Fisher, USA). The raw sequences of the COI, 16s rRNA and Rhodopsin (rod) gene regions were arranged and aligned using the ClustalW algorithm (Thompson *et al.* 1994) in BioEdit v.7.2.5 (Hall 1999). Low quality sequences were discarded. Species identification was performed by comparing sequence similarity with the reference dataset (Wong and Hanner 2008). Separation regions, haplotype number, nucleotide diversity (π) and haplotype diversity (Hd) and Tajima D statistics were used for populations by DnaSP v.5.0 (Librado and Rozas 2009). FST pairwise values and genetic heterogeneity were generated using ARLEQUIN version 5 (Schneider *et al.* 2000). The haplotype distribution was created using the PopART programme (Leigh and Bryant 2015).

3 | RESULTS

In this research, the morphometric and meristic characters were counted and calculated for female and male samples shown in Table 1 and 2. Atlantic bonito has 20 – 22 fin rays in the first dorsal fin and 14 – 16 dorsal fins in the second fin rays, 23 – 25 fin rays in pectoral fins, 6 – 7 fin rays in pelvic fins and 14 – 15 fin rays in the anal fin. Scale numbers in the lateral line varied between 400 and 500, vertebral bones were counted as 56 – 62, and gill rakers were counted as 14 – 15 in the first raker and 18 – 19 in the second one and 16 – 20 in the last one. The Atlantic bonito has finlets on each side of the leading to the forked caudal fin.

Table 1 Meristic counts of Atlantic bonito *Sarda sarda*.

Meristic characters	Number
Vertebral counts	56 – 62
First dorsal fin rays	20 – 22
Second dorsal fin rays	14 – 16
Anal fin rays	14 – 15
Pectoral fin rays	23 – 25
Pelvic fin rays	6 – 7
Gill rakers	I/14-15, II/18-19, III/16-20
Scale along lateral line	400-500
Dorsal finlets	5 – 8
Anal finlets	5 – 7

The ratio of the standard length, pre-dorsal length, pre-anal length, pre-pelvic length, dorsal fin based body

height, anal fin based body height, the first and second dorsal fin length, pectoral fin length, caudal fin upper and down lob length, head length, nostril length, the distance between nostrils, the distance between eyes, mouth length and mouth width in females were greater or bigger than male. By contrast, fork length, pelvic fin length, anal fin length and orbital length were higher in male than female (*t*-test; $p < 0.05$). However, there were no significant differences between other characteristics of male and female (*t*-test; all $p > 0.05$).

As a result of the genetic analysis, 10 haplotypes were determined for COI gene region, 3 haplotypes for 16s gene region and 4 haplotypes for the rhodopsin gene region in three sampling areas (Figures 2 – 4). The haplotype sequences of the gene regions were uploaded to the NCBI (Accession numbers: COI, MW279200- MW279210; 16s, MW130119- MW130121). The most common haplotypes of COI (H2), 16s rRNA (H1), Rhod (H1) were shared by specimens from samplings. The genetic diversity parameters of each sampling site for each mtDNA and nuclear marker are given in Table 3. Pairwise FST values ranged between –0.05045 and 0.03848 for the COI gene region, 0.00000 and 0.14985 for the 16s gene region, 0.00000 and 0.07386 for the Rhod gene region. While the FST value for Tirebolu population yielded as statistically significant for 16s and Rhod gene region, it is insignificant for other populations. FST values were found for all populations yielded as statistically insignificant (Tables 4) for the COI gene region. Tajima's *D* test, which indicates neutrality, showed statistical significance for COI gene regions and population balancing selection or sudden population contraction (Table 5).

TABLE 2 Morphometric measurements (mm) of Atlantic bonito *Sarda sarda* (TL, total length; FL, fork length; SL, standard length; PAL, pre-anal length; PDL, pre-dorsal length; PPL, pre-pelvic length; BHD, dorsal fin based body height; BHA, anal fin based body height; DFL1, first dorsal fin length; DFL2, second dorsal fin length; PCFL, pectoral fin length; PVFL, pelvic fin length; AFL, anal fin length; CFUL, caudal fin upper lob length; CFDL, caudal fin down lob length; HL, head length; NL, nostril length; DN, distance between nostrils; OL, orbital length; DE, distance between eyes; ML, mouth length; MW, mouth width).

Character	Female (♀; *n* = 60)			Male (♂; *n* = 60)			*p-value*
	$\bar{x}$±SE	Range	TL (%)	$\bar{x}$±SE	Range	TL (%)	
TL	270.44 ± 9.38	175.0 – 373.03		261.35 ± 13.64	177.0 – 380.0		0.293
FL	246.54 ± 9.86	165.0 – 345.0	91.17	243.33 ± 12.28	168.0 – 344.0	93.11	0.419
SL	231.35 ± 7.89	153.0 – 318.0	85.55	222.25 ± 11.57	150.0 – 321.0	85.04	0.259
PAL	162.64 ± 5.53	107.79 – 223.39	60.14	156.11 ± 8.17	106.00 – 227.57	59.89	0.269
PDL	67.67 ± 2.25	44.66 – 92.56	25.02	64.85 ± 3.38	43.92 – 94.29	24.81	0.246
PPL	72.35 ± 2.47	48.02 – 99.52	26.75	69.73 ± 3.64	47.22 – 101.38	26.68	0.277
BHD	45.71 ± 1.54	30.26 – 62.72	16.90	43.94 ± 2.29	29.76 – 63.89	16.81	0.263
BHA	37.89 ± 1.29	25.12 – 52.05	14.01	36.47 ± 1.90	24.70 – 53.02	13.95	0.269
DFL1	61.99 ± 3.73	34.74 – 117.35	22.92	59.17 ± 4.78	35.14 – 109.40	22.64	0.322
DFL2	29.82 ± 0.85	18.60 – 38.45	11.03	28.29 ± 1.26	17.02 – 38.21	10.83	0.160
PCFL	25.33 ± 0.87	15.73 – 34.87	9.37	24.43 ± 1.28	16.54 – 35.52	9.35	0.281
PVFL	19.81 ± 0.69	13.15 – 26.56	7.33	19.19 ± 1.01	13.00 – 27.90	7.34	0.306
AFL	20.51 ± 0.71	13.65 – 28.30	7.58	19.83 ± 1.03	13.43 – 28.82	7.59	0.294
CFUL	41.66 ± 1.66	26.33 – 61.89	15.41	39.45 ± 2.18	26.63 – 60.21	15.09	0.211
CFDL	40.02 ± 1.49	25.74 – 59.23	14.79	38.25 ± 2.02	26.03 – 57.14	14.64	0.243
HL	64.16 ± 2.17	42.28 – 88.12	23.73	61.74 ± 3.22	41.81 – 89.76	23.62	0.268
			HL (%)			**HL (%)**	
NL	21.40 ± 0.71	14.49 – 30.53	33.35	20.47 ± 0.94	14.25 – 28.99	33.15	0.217
DN	11.71 ± 0.49	7.30 – 30.53	18.25	11.20 ± 0.67	7.38 – 18.83	18.15	0.273
OL	11.36 ± 0.46	6.85 – 16.08	17.70	11.27 ± 0.59	7.63 – 16.38	18.25	0.452
DE	16.37 ± 0.44	10.99 – 21.57	25.51	15.11 ± 0.79	10.29 – 21.97	24.48	0.086
ML	34.78 ± 1.19	22.57 – 50.26	54.20	32.92 ± 1.62	22.83 – 48.46	53.32	0.179
MW	21.87 ± 0.49	14.69 – 34.61	34.09	20.29 ± 0.92	14.45 – 28.34	32.86	0.096

TABLE 3 Genetic diversity parameters of four sampling sites of *Sarda sarda* based on mtDNA sequence data.

Collection site	COI					RHOD					16S				
	n	*H*	H_d	*PS*	π	*n*	*H*	H_d	*PS*	π	*n*	*H*	H_d	*PS*	π
TRABZON	34	9	0,845	17	0,00984	39	4	0,317	3	0,00105	29	3	0,352	2	0,00083
TIREBOLU	25	6	0,760	16	0,01089	30	1	0,000	0	0,000	28	1	0,000	0	0,000
SINOP	11	4	0,491	15	0,00990	12	1	0,000	0	0,000	14	1	0,000	0	0,000

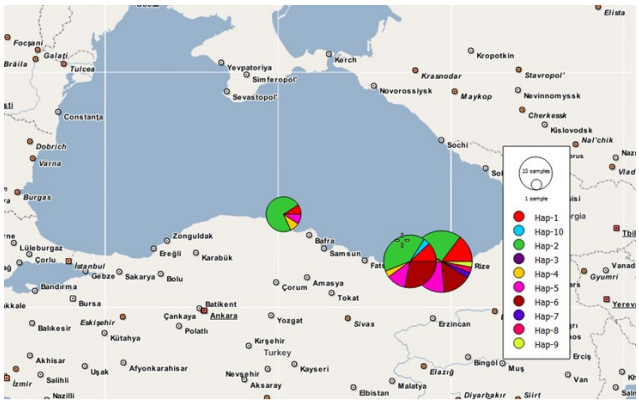

FIGURE 2 Distribution of haplotypes of cytochrome c oxidase subunit I (COI) gene region seen in *Sarda sarda* populations.

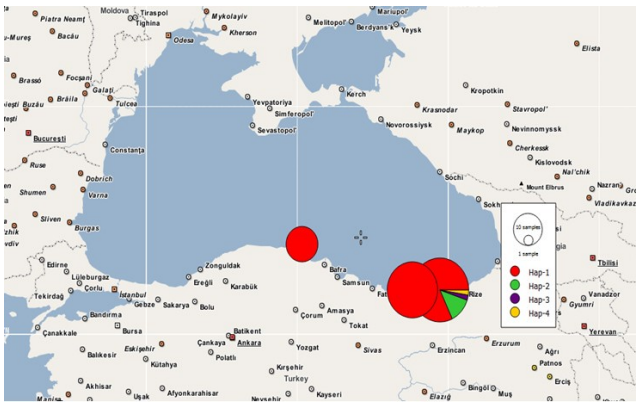

FIGURE 3 Distribution of haplotypes of Rhodopsin (rod) gene region seen in *Sarda sarda* populations.

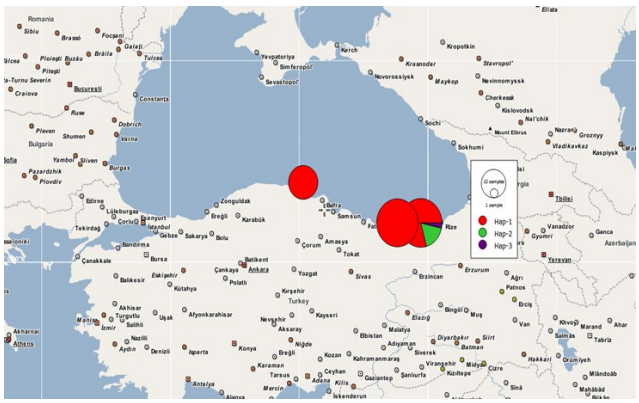

FIGURE 4 Distribution of haplotypes of 16s rRNA gene region seen in *Sarda sarda* populations.

4 | DISCUSSION

This study demonstrates morphometric, meristic and genetic characteristics of Atlantic bonito. The number of spines in the first dorsal fin was counted as 20 – 22 which are similar to the findings of Collette and Chao (1975) but lower than Demir (1964). The number of pectoral fin ray recorded was also similar to the findings of Collette and Chao (1975) but not for the vertebral count and gill rakers. The number of finlets recorded in our study was found different in other studies (Demir 1964; Collette and Chao 1975; Collette and Nauen 1983).

TABLE 4 Pairwise FST values between three populations of *Sarda sarda* for studied genes.

Gene	Locality	Trabzon	Tirebolu	Sinop
Rhodopsin	Trabzon	0.00000		
	Tirebolu	0.07386	0.00000	
	Sinop	0.02477	0.00000	0.00000
COI	Trabzon	0.00000		
	Tirebolu	0.03028	0.00000	
	Sinop	0.03848	–0.05045	0.00000
16s	Trabzon	0.00000		
	Tirebolu	0.14985	0.00000	
	Sinop	0.09470	0.00000	0.00000

TABLE 5 Neutrality statistics of *Sarda sarda* populations.

Gene	Neutrality Test
COI	Tajima's *D*: 2,96100
	Statistical significance: **, $p < 0.01$
16S rRNA	Tajima's *D*: –0,91038
	Statistical significance: $p > 0.10$
Rhodopsin	Tajima's *D*: –1,13613
	Statistical significance: $p > 0.10$

Most of the morphometric characters in female were longer than male, similar to the findings of Franicevic *et al.* (2005). The present study is the first comprehensive study that shows sex-based morphometric measurements of Atlantic bonito from the Turkish waters. The variation in morphometric and meristic characters of this species from different locations could be due to varying environmental conditions and genetic biodiversity (Collette and Chao 1975; Franicevic *et al.* 2005).

There are limited genetic studies on the economically important pelagic species; especially those are bigger in size. Therefore, we investigated Atlantic bonito's genetic characterisation within the concept of national registration of the fish species by the Ministry of Agriculture and Forestry. Yoshida (1980) found varying meristic and morphometric differences between and among populations of Atlantic bonito in five sampling areas. For instance, the meristic characters were similar for the two Western Atlantic populations as they were for the Mediterranean and Gulf of Guinea populations. Study of Turan *et al.* (2015), based on the analysis of D-loop gene region for Atlantic bonito populations, revealed that the Marmara and Black Sea populations of Atlantic bonito was similar whereas and the Aegean and Mediterranean populations were different. Similar results using a microsatellite marker were also obtained by Turan (2015). Our study, based on three different gene regions, may be considered better to clarify the genetic structure of Atlantic bonito populations. Among these genes, COI and 16s rRNA regions are two gene regions that are frequently used in

speciation research (McDowell and Graves 2002; Hyde *et al.* 2005; Lopez and Pardo 2005; Paine *et al.* 2007). On the other hand, rhodopsin nuclear gene region, which reflects a basic mechanism for ecological processes such as speciation and local adaptation, is used in phylogenetic research (Ebert and Andrew 2009; Sivasundar and Palumbi 2010; Shum *et al.* 2014).

In conclusion, the morphometric and meristic as well as genetic identification of a species is crucial for its management. This study will be helpful to determine the management strategy of a species with high economic value such as the Atlantic bonito.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Sİ & ZDD data collection and analysis. NK data analysis and preparation of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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